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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/EP97/05079 (22) International Filing Date: 17 September 1997 (17.09.97) (30) Priority Data: MI96A001919 18 September 1996 (18.09.96) IT MI96A001920 18 September 1996 (18.09.96) IT MI96A001921 18 September 1996 (18.09.96) IT MI96A001922 18 September 1996 (18.09.96) IT (71) Applicant (for all designated States except US): ZETESIS S.P.A. [IT/IT]; Galleria del Corso, 2, I-20122 Milano (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): PANERAI, Alberto, E. [IT/IT]; Galleria del Corso, 2, I-20122 Milano (IT). BAR-TORELLI, Alberto [IT/IT]; Galleria del Corso, 2, I-20122 Milano (IT). (74) Agent: SPADARO, Marco; Studio Consulenza Brevettuale, Via Rossini, 8, I-20122 Milano (IT).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: USE OF PROTEINS AS AGENTS AGAINST AUTOIMMUNE DISEASES (57) Abstract <p>The use of proteins extracted with perchloric acid from animal organs, for the preparation of medicaments active against autoimmune diseases, in particular with activity against atherosclerosis, arthritis, multiple sclerosis, diabetes.</p>		

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USE OF PROTEINS AS AGENTS AGAINST AUTOIMMUNE DISEASES

The present invention relates to the use of proteins extractable from animal organs, particularly from livers of mammals, for the preparation of medicaments active against autoimmune diseases, in particular activity against atherosclerosis, arthritis, multiple sclerosis, diabetes.

The administration of complete Freund's adjuvant has proved to be capable of inducing an experimental arthritis very similar to rheumatoid arthritis in rats. On the other hand, the administration of adjuvant to rabbits induces no arthritic pathology, but atherosclerosis. The studies carried out have evidenced that, in both lesions, immunoreactivity to an endogenous factor, which has been identified as the Heat Shock Protein 60 (HSP60), is present. Subsequent searches have confirmed these observations, proving that the administration of complete Freund's adjuvant can be replaced by the administration of HSP60, resulting in the same pathologies. Afterwards, pre-treatment of rat with adjuvant, HSP60 or fragments thereof has proved to prevent the onset of arthritis, with a still obscure mechanism, whereas the administration subsequent to the adjuvant worsens the progress of the disease.

More recently, pre-treatment with adjuvant has been found to also prevent other experimental pathologies which can be defined, generally speaking, as autoimmune disease, such as diabetes or experimental allergic encephalomyelitis (EAE). Finally, HSP60 has been found to have structural analogies to a high number of

autoantigens, therefore it is assumed to be related to pathologies more widely than what up to now observed.

WO 92/10197 disclosed protein fractions extractable with perchloric acid from organs of mammals, and their use as anticancer agents. Within these fractions, three main components could be identified, having molecular weights 50, 14 and 10 KDa on gel electrophoresis. The purified extract containing these three components will be referred to as UK 101 in the following. The sequence of the 14 KDa protein component, which is the main, if not the only, responsible for the described activities, is reported in the Table hereinbelow and in WO 96/02567, and it has turned out to be related to that described by other authors (Levy-Favatier, Eur. Biochem. 1903, 212 (3) 665-73) which have assumed that the novel identified sequences belong to the family of the proteins known as chaperonins, to which the HSPs themselves belong.

The proteins described in WO 92/10197 and those of WO 96/02567 (in the following referred to as UK 114) show anyhow properties never observed for chaperonins or analogous proteins. More specifically, it has been found that said proteins can be used in the prevention and in the treatment of autoimmune diseases, in particular atherosclerotic conditions, such as the atherosclerosis induced by organ transplants, arthritis, multiple sclerosis and diabetes.

The invention relates preferably to the use of the purified proteins UK 101 and UK 114 for the preparation of medicaments for the prevention and the treatment of autoimmune diseases such as atherosclerosis following organ transplants, arthritis, multiple sclerosis,

diabetes.

Moreover the invention comprises the use of proteins showing a high homology degree to UK 114, of at least 80%, preferably of at least 90%.

5 **ANTIATHEROSCLEROTIC ACTIVITY**

It has been ascertained that nowadays the more frequent cause of failure of organ transplants in time is no more the rejection, but the formation of atherosclerotic plaques at the contact point between the
10 vases of the transplanted organ and those of the host. This pathology is worsened by the usual immunosuppressors such as cyclosporin, whereas the use of AZT, which is however very toxic, appears to be useful.

15 The activity of the proteins UK 101 and UK 114 has been evidenced using both a conventional atherosclerosis model, which is that of the rabbit pre-treated with complete Freund's adjuvant, and a transplant atherosclerosis model. In the first case, the
20 subcutaneous treatment with adjuvant induces within 21 days the formation of atherosclerotic plaques at the iliac bifurcation and at the aortic arch. The pre-treatment (7 days before) with UK 101 or UK 114 has significantly prevented the development of the pathology
25 in a high percent of cases compared with the treatment with the only adjuvant, which has lead to the development of the disease in all of the animals.

On the other hand, the experimental model of transplant atherosclerosis consists in the venous by-
30 pass s at the level of arteri s in the rat. After a short time, the formation of atherosclerotic plaques at

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the level of the host vase, as it happens in the human pathology, has been observed. The pre-treatment (7 days before) with UK 101 or UK 114 has significantly prevented the development of the pathology in a high percent of cases, compared with what observed in the animals non pre-treated before the transplant.

ANTIARTHRITIS ACTIVITY

This activity has been evidenced using a conventional arthritis model, which is the adjuvant-induced arthritis. In this model, Lewis rats are injected at the tail base with complete Freund's adjuvant: within 7 days, a pathology at the rear leg appears, characterized by swelling and joints alterations. The pathology reaches its peaks from the 14th to the 21st day, then decreasing until the leg returns to normal conditions. The pre-treatment (7 days before) with UK 101 or UK 114 has significantly prevented the development of the pathology in a high percent of cases compared with treatment with the only adjuvant, which has lead to the development of the pathology in 100% of the animals. The treatment with UK 101 or UK 114 after the administration of adjuvant has worsened the progress of the pathology.

Therefore, it is considered that UK 101 and UK 114 are capable of modifying the progress of or of preventing pathological conditions such as arthritis and rheumatoid arthritis.

ACTIVITY AGAINST MULTIPLE SCLEROSIS

This has been evidenced using a conventional multiple sclerosis model: the experimental allergic encephalomyelitis (EAE). The pathology is induced

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injecting subcutaneously Lewis rats with a Guinea-pig spinal cord homogenate together with complete Freund's adjuvant. The pathology appears as a progressive paralysis starting from the rear limbs, which begins at about the 12th day, reaches a maximum at about the 21st day and undergoes remission at about the 30th day from the administration of the immunogen. The pre-treatment (7 days before) with UK 101 or UK 114 has significantly prevented the development of the pathology in a high percent of cases and a less serious pathology has appeared, compared with treatment with the only marrow homogenate and adjuvant, which has lead to the development of the pathology in 100% of the animals.

Therefore UK 101 and UK 114 are believed to be able of changing the progress of or preventing pathological conditions such as multiple sclerosis.

ANTIDIABETIC ACTIVITY

This has been evidenced using a conventional diabetes model, represented by the BB rat which spontaneously develops diabetes around the 45th day of life. The animals have been treated at the 30th day of life with UK 101 or UK 114 and the development of the pathology has been observed, compared with untreated control animals. The pre-treatment has been found to decrease the incidence and the severity of the pathology in the experimental model. Some patients affected with tumors at different sites and also suffering from diabetes have been treated with UK 101 in the course of a compassionate treatment with the substance. All of the patients treated, independently of the effect on the tumor pathology, have shown a remission of the diabetic

pathology going so far as to quit the insulin therapy.

Therefore UK 101 and UK 114 are believed to be capable of changing the course of diabetes or of preventing it.

5 The antidiabetic activity has in fact been confirmed, although up to now in a limited number of cases, also in vivo in patients suffering from diabetes.

The proteins of the invention can be administered using suitable formulations, mainly injectable.

10 The pattern of the administration (doses, frequency of administration, etc.) will be determined according to the circumstances, depending on factors such as conditions of the patient, phase of the disease, etc., but usually a daily dosage ranging from 1 to 100 mg will
15 be suitable.

TABLE

	Met	Ser	Glu	Asn	Ser	Glu	Glu	Pro	Val	Gly	Glu	Ala	Lys	Ala
	1				5					10				
20	Pro	Ala	Ala	Ile	Gly	Pro	Tyr	Ser	Gln	Ala	Val	Leu	Val	Asp
	15					20					25			
	Arg	Thr	Ile	Tyr	Ile	Ser	Gly	Gln	Leu	Gly	Met	Asp	Pro	Ala
		30					35					40		
	Ser	Gly	Gln	Leu	Val	Pro	Gly	Gly	Val	Val	Glu	Glu	Ala	Lys
			45					50					55	
25	Gln	Ala	Leu	Thr	Asn	Ile	Gly	Glu	Ile	Leu	Lys	Ala	Ala	Gly
				60					65					70
	Cys	Asp	Phe	Thr	Asn	Val	Val	Lys	Ala	Thr	Val	Leu	Leu	Ala
					75					80				
30	Asp	Ile	Asn	Asp	Phe	Ser	Ala	Val	Asn	Asp	Val	Tyr	Lys	Gln
	85					90					95			
	Tyr	Phe	Gln	Ser	Ser	Phe	Pro	Ala	Arg	Ala	Ala	Tyr	Gln	Val
		100					105					110		
	Ala	Ala	Leu	Pro	Lys	Gly	Gly	Arg	Val	Glu	Ile	Glu	Ala	Ile
			115					120					125	
35	Ala	Val	Gln	Gly	Pro	Leu	Thr	Thr	Ala	S	r	Val		
				130					135					

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT:

(A) NAME: zetesis s.p.a.

(B) STREET: Galleria del Corso 2

(C) CITY: Milano

(E) COUNTRY: Italy

10 (F) POSTAL CODE (ZIP): 20122

(ii) TITLE OF INVENTION: Use of proteins as agents
against autoimmune diseases

15 (iii) NUMBER OF SEQUENCES: 1

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

20 (C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version
#1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 137 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

30 (D) TOPOLOGY: linear

8

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

5 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

10	Met	Ser	Glu	Asn	Ser	Glu	Glu	Pro	Val	Gly	Glu	Ala	Lys	Ala
	1				5					10				
	Pro	Ala	Ala	Ile	Gly	Pro	Tyr	Ser	Gln	Ala	Val	Leu	Val	Asp
	15				20						25			
	Arg	Thr	Ile	Tyr	Ile	Ser	Gly	Gln	Leu	Gly	Met	Asp	Pro	Ala
		30					35					40		
15	Ser	Gly	Gln	Leu	Val	Pro	Gly	Gly	Val	Val	Glu	Glu	Ala	Lys
			45				50						55	
	Gln	Ala	Leu	Thr	Asn	Ile	Gly	Glu	Ile	Leu	Lys	Ala	Ala	Gly
				60					65					70
20	Cys	Asp	Phe	Thr	Asn	Val	Val	Lys	Ala	Thr	Val	Leu	Leu	Ala
					75					80				
	Asp	Ile	Asn	Asp	Phe	Ser	Ala	Val	Asn	Asp	Val	Tyr	Lys	Gln
	85					90					95			
	Tyr	Phe	Gln	Ser	Ser	Phe	Pro	Ala	Arg	Ala	Ala	Tyr	Gln	Val
		100					105					110		
25	Ala	Ala	Leu	Pro	Lys	Gly	Gly	Arg	Val	Glu	Ile	Glu	Ala	Ile
			115					120					125	
	Ala	Val	Gln	Gly	Pro	Leu	Thr	Thr	Ala	Ser	Val			
				130					135					

CLAIMS

1. The use of proteins extractable with perchloric acid from mammal liver, for the preparation of medicaments active against autoimmune diseases.

2. The use according to claim 1, wherein the protein has the following sequence:

	Met	Ser	Glu	Asn	Ser	Glu	Glu	Pro	Val	Gly	Glu	Ala	Lys	Ala
	1				5					10				
10	Pro	Ala	Ala	Ile	Gly	Pro	Tyr	Ser	Gln	Ala	Val	Leu	Val	Asp
	15				20						25			
	Arg	Thr	Ile	Tyr	Ile	Ser	Gly	Gln	Leu	Gly	Met	Asp	Pro	Ala
		30					35					40		
15	Ser	Gly	Gln	Leu	Val	Pro	Gly	Gly	Val	Val	Glu	Glu	Ala	Lys
			45				50						55	
	Gln	Ala	Leu	Thr	Asn	Ile	Gly	Glu	Ile	Leu	Lys	Ala	Ala	Gly
				60					65					70
	Cys	Asp	Phe	Thr	Asn	Val	Val	Lys	Ala	Thr	Val	Leu	Leu	Ala
					75					80				
20	Asp	Ile	Asn	Asp	Phe	Ser	Ala	Val	Asn	Asp	Val	Tyr	Lys	Gln
	85					90					95			
	Tyr	Phe	Gln	Ser	Ser	Phe	Pro	Ala	Arg	Ala	Ala	Tyr	Gln	Val
		100					105					110		
25	Ala	Ala	Leu	Pro	Lys	Gly	Gly	Arg	Val	Glu	Ile	Glu	Ala	Ile
			115				120						125	
	Ala	Val	Gln	Gly	Pro	Leu	Thr	Thr	Ala	Ser	Val			
				130					135					

3. The use according to claim 1, wherein the proteins used have a homology of at least 80% to the protein of claim 2.

4. Pharmaceutical compositions containing as the active ingredient the proteins of claims 1-3 in admixture with suitable excipients.

5. The use according to claim 1, for the preparation of medicaments for the prevention and the treatment of atherosclerosis following transplants.

6. The use according to claim 1, for the preparation

10

of medicaments for the prevention and the treatment of arthritis.

7. The use according to claim 1, for the preparation of medicaments for the prevention and the treatment of multiple sclerosis.

8. The use according to claim 1, for the preparation of medicaments for the prevention and the treatment of diabetes.

INTERNATIONAL SEARCH REPORT

Int. onal Application No

PCT/EP 97/05079

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6. A61K38/17 C07K14/47

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 02567 A (ZETESIS SPA ;RONCHI SEVERINO (IT); BARTORELLI ALBERTO (IT)) 1 February 1996 see the whole document ---	4
X	WO 92 10197 A (BARTORELLI ALBERTO ;TURIANO ANGELA (IT)) 25 June 1992 see the whole document ---	4
X	T. OKA ET AL: "Isolation and characterisation of novel perchloric acid-soluble protein inhibiting cell-free protein synthesis" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 270, no. 50, 15 December 1995, MD US, pages 30060-30067, XP002053817 see the whole document ---	4
-/-		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

29 January 1998

Date of mailing of the international search report

18/02/1998

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European Patent Office, P.B. 5818 Patentaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Cervigni, S

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/05079

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEVY-FAVATIER F ET AL: "CHARACTERIZATION, PURIFICATION AND CDNA CLONING OF A RAT PERCHLORIC-ACID-SOLUBLE 23-KDA PROTEIN PRESENT ONLY IN LIVER AND KIDNEY" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 212, no. 3, March 1993, pages 665-673, XP000673794 see the whole document ----	4
X	WO 93 18146 A (INST NAT SANTE RECH MED) 16 September 1993 see the whole document see page 11, line 14 - line 23 ----	1,5-8
X	US 4 024 247 A (FORTINI JACK G ET AL) 17 May 1977 see the whole document -----	1,5-8

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/EP 97/05079

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9602567 A	01-02-96	IT 1270618 B AU 3077995 A CA 2194861 A CZ 9700069 A EP 0770093 A FI 970097 A HU 76328 A NO 970114 A ZA 9505837 A	07-05-97 16-02-96 01-02-96 13-08-97 02-05-97 06-03-97 28-08-97 05-03-97 21-02-96
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US 4024247 A	17-05-77	US 3876774 A AU 1269976 A BE 840435 A CA 1066620 A DE 2614833 A FR 2306708 A GB 1529890 A JP 51133407 A NL 7603572 A	08-04-75 13-10-77 02-08-76 20-11-79 21-10-76 05-11-76 25-10-78 19-11-76 11-10-76